RESEARCH ARTICLE

SABRAO Journal of Breeding and Genetics
57 (2) 740-751, 2025
http://doi.org/10.54910/sabrao2025.57.2.30
http://sabraojournal.org/
pISSN 1029-7073; eISSN 2224-8978

MODIFIED BIOCHAR'S ROLE IN INCREASING RICE (*ORYZA SATIVA* L.) PRODUCTION WITH REDUCED MERCURY CONTENT IN MERCURY-CONTAMINATED SOILS

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SUMMARY

The reduction of mercury uptake and its content in rice (*Oryza sativa* L.) grains is this research's aim, particularly on rice grown in mercury-contaminated fields in the Mandailing Natal Regency, Indonesia. The experiment, laid out in a split-split plot design, had three replications and the period from August to December 2023. The main plots included biochar (from coconut shells) modified with Fe3O4 (M1), FeSO4 (M2), dolomite (M3), and unmodified biochar (M0). The subplots were pyrolysis temperatures at two levels, 350 °C (S1) and 550 °C (S2). The sub-sub plots comprised three rice cultivars: IF-16, Inpari-32, and Ciherang. Additionally, the study included three control treatments (without biochar). The results showed biochar modifications with Fe3O4, FeSO4, and dolomite can reduce the mercury content in rice grains below the standard quality threshold. Among the treatments, the IF-16 cultivar combined with biochar modified with Fe3O4 was the most effective in reducing mercury content while significantly increasing production in mercury-contaminated soils. This combination led to an 80.1% increase in rice production with lower mercury content in the grains.

Keywords: Rice (*O. sativa* L.), food, gold mine, heavy metals, Hg, tolerance

Key findings: The results showed rice (*O. sativa* L.) tolerant cultivar IF-16, combined with modified biochar, can significantly reduce the mercury content in rice grains compared with cultivars Ciherang and Inpari-32 grown by the farming community on mercury-contaminated soils.

Communicating Editor: Prof. Naqib Ullah Khan

Manuscript received: June 23, 2024; Accepted: September 29, 2024.

Citation: Triyanti VR, Rosmayati, Basyuni M, Damanik RIM (2025). Modified biochar's role in increasing rice (*Oryza sativa* L.) Production. *SABRAO J. Breed. Genet.* 57(2): 740-751. http://doi.org/10.54910/sabrao2025.57.2.30.

INTRODUCTION

Mandailing Natal Regency, North Sumatra, Indonesia, with a total area of 662,070 hectares, has 17,159 hectares of rice fields, producing 72,323 tons of rice (BPS, 2022). In this region, 43.08% of the 221,126 workforce participate in rice (*O. sativa* L.) production, primarily for personal consumption, with the remainder sold. The primary water source for irrigation in this area is the Batang Gadis River, which also provides drinking water and food resources like fish.

However, the unlicensed gold mining operations, known as PETI (Penambangan Emas Tanpa Izin), particularly in the districts of Muara Sipongi, Hutapungkut, Hutabargot, and Naga Juang, discharge mercury wastes into the Batang Gadis River through the amalgamation process. This pollution is harmful to both the environment and public health (WHO, 2021).

Rice fields irrigated with water contaminated by the mercury waste have shown its alarming levels. In Hutabargot District, for example, mercury levels in rice fields have reached 14.26 ppm (Syahril *et al.,* 2024), far exceeding the quality standard threshold of 0.03 mg kg^{-1} (BPOM, 2018). Similar contamination levels were evident in Naga Juang District, with 13.26 ppm reported by Triyanti *et al.* (2024).

Mercury contamination in rice production is a significant health risk, as rice is a major source of mercury exposure for billions of people globally. Studies have shown rice contributing to 94%–96% of total mercury intake in humans, while fish contributes only 1%–2% (Giwa *et al.,* 2022). Mercury levels in rice grains can reach as high as 569 µg kg^{-1} (Zhang *et al.,* 2019), particularly in regions near mercury mining areas (Wu *et al.,* 2018).

Bioremediation, specifically through biochar, has been a proposed solution to reduce mercury contamination (Tiodar *et al.,* 2021). Biochar, produced from organic materials, has shown to adsorb and stabilize mercury in soil and water (Gomez-Eyles *et al.,* 2013). Studies have demonstrated various types of biochar can reduce mercury uptake in plants, including rice (Shu *et al.,* 2016; Giwa *et al.,* 2022), and arsenic adbsortion with sulfur enriched biochar (Fan *et al.,* 2013).

The efficacy of biochar in reducing mercury can vary based on its preparation method, particularly the pyrolysis temperature, which affects its physical and chemical properties (Cox *et al.,* 2012; Enaime *et al.,* 2020; Zhao *et al.,* 2020). Kan *et al.* (2016) classified biochar preparation into conventional, fast, and ultra-fast pyrolysis, each with different effects on mercury reduction.

This study aimed to evaluate the use of modified biochar derived from coconut shells, an abundant resource in Mandailing Natal Regency, in combination with three rice cultivars (IF-16, Inpari-32, and Ciherang) with different tolerances to mercury stress. The goal is to reduce mercury uptake in rice plants and ensure mercury content in rice grains remains below the safety threshold.

MATERIALS AND METHODS

Experimental procedure

The presented study commenced in rice (*O. sativa* L.) fields in Mandailing Natal Regency, located at an altitude of ±300 m above sea level, from August to December 2023 (Figure 1). The materials used in this study included HNO3 PA, H2SO4 PA, 30% H2O2 solution, and three rice cultivars: IF-16 (tolerant), Inpari-32 (moderate), and Ciherang (sensitive). The classification of rice genotypes as tolerant, moderate, or sensitive relied on morphological parameters and molecular analyses (Triyanti *et al.,* 2024). The equipment used included a UV-Vis spectrophotometer, glassware, measuring flasks, and other tools required for mercury (Hg) analysis.

Experimental design

An employed split-split plot design had the biochar modification as the main factor. The four types of biochar were: Fe3O4-modified biochar (M1), FeSO4-modified biochar (M2), dolomite-modified biochar (M3), and

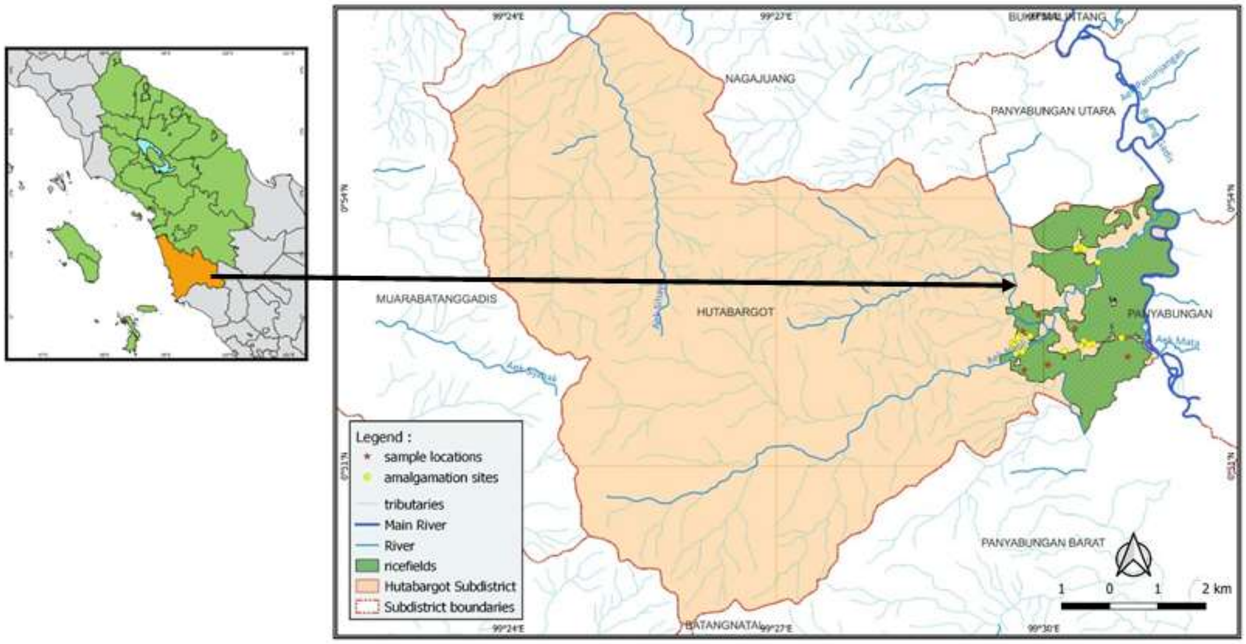

Figure 1. Overview of the study site and amalgamation sites.

unmodified biochar (M0). The second factor (subplots) was pyrolysis temperature, with two levels: 350 °C (S1) and 550 °C (S2). The third factor (sub-subplots) consisted of the three rice cultivars (V1 = IF-16, V2 = Inpari-32, and V3 = Ciherang). Additionally, the research included three control treatments without biochar. In total, 27 experimental units comprised the trials, each measuring 2 m × 1 m, with three replications. The biochar used came from coconut shells.

Mercury content measurement

The measurement of mercury content in rice grains and soil had the collected samples digested using a mixture of concentrated HNO_3 and H_2SO_4, followed by H_2O_2, to break down organic matter. The digested samples underwent analysis using a UV-Vis spectrophotometer to quantify mercury levels. Calibration of the spectrophotometer employed standard mercury solutions to ensure accuracy in the measurements. Monitoring the detection limits and recovery rates helped maintain the reliability of the mercury content data.

Study of traits and analysis

The parameters observed were the number of tillers, flowering age, harvest age, number of empty grains, number of filled grains, production per subplot, and Hg content in rice roots, stems, leaves, and grains. The Hg content in roots, stems, leaves, and grains bore analysis from destructive samples. For the extraction of Hg from plant tissues, the study followed the method of Du *et al.* (2021). The measurement of Hg metal content progressed by using the UV-Vis spectrophotometer with the addition of dithizonate at a wavelength of 495 nm (Ahmad *et al.,* 2001; Zaetun *et al.,* 2015). Upon compiling the data obtained from the spectrophotometer, their interpretation engaged a simple linear regression equation obtained from the blank sample that either has no Hg or has very low Hg concentrations. By using this simple linear regression equation, interpreting the data obtained from the spectrophotometer ensures accurate calculation of the Hg content in rice plant samples (Ng *et al.,* 2018).

Table 1. Number of tillers in three rice cultivars with biochar modified treatments.

Biochar modifications	Pyrolysis Temperature	Cultivars		
		IF-16	Inpari-32	Ciherang
	2 Weeks After Planting (stems)			
Control (without biochar)		2.73d	2.93d	3.03d
Biochar	350 °C	3.40bc	3.67abc	4.00abc
Biochar	550 °C	3.40bc	4.10abc	4.30ab
Biochar + Fe_3O_4	350 °C	3.93abc	3.43bc	3.47bc
Biochar + Fe_3O_4	550 °C	3.93abc	4.00abc	3.70abc
Biochar + $FeSO_4$	350 °C	3.97abc	3.20c	3.33c
Biochar + $FeSO_4$	550 °C	3.93abc	3.33c	4.53a
Biochar + Dolomite	350 °C	3.57bc	4.03abc	3.97abc
Biochar + Dolomite	550 °C	3.60bc	3.57bc	3.37c
	4 Weeks After Planting (stems)			
Control (without biochar)		6.53b	6.77b	6.70b
Biochar	350 °C	7.53a	7.80a	7.57a
Biochar	550 °C	7.57a	7.60a	7.63a
Biochar + Fe_3O_4	350 °C	7.37a	7.57a	7.77a
Biochar + Fe_3O_4	550 °C	7.80a	7.57a	7.53a
Biochar + $FeSO_4$	350 °C	7.67a	7.67a	7.47a
Biochar + $FeSO_4$	550 °C	7.50a	7.90a	7.57a
Biochar + Dolomite	350 °C	7.50a	7.83a	7.53a
Biochar + Dolomite	550 °C	7.40a	7.70a	7.80a
	6 Weeks After Planting (stems)			
Control (without biochar)		10.13k	10.87k	8.83l
Biochar	350 °C	13.90hij	16.53a	15.20b-g
Biochar	550 °C	15.20b-g	16.17ab	14.40g-j
Biochar + Fe_3O_4	350 °C	13.47j	15.23b-g	14.50f-j
Biochar + Fe_3O_4	550 °C	15.70a-f	15.73a-e	16.20ab
Biochar + $FeSO_4$	350 °C	13.80ij	15.87a-d	16.53a
Biochar + $FeSO_4$	550 °C	15.90a-d	14.60e-i	14.70d-i
Biochar + Dolomite	350 °C	13.77j	16.03abc	14.90c-i
Biochar + Dolomite	550 °C	15.83a-d	15.03b-h	15.77a-e
	8 Weeks After Planting (stems)			
Control (without biochar)		16.87g	15.57h	12.47i
Biochar	350 °C	19.57def	20.20de	19.57def
Biochar	550 °C	25.03a	20.47d	24.87a
Biochar + Fe_3O_4	350 °C	25.23a	24.53ab	19.47def
Biochar + Fe_3O_4	550 °C	24.70a	24.93a	25.10a
Biochar + $FeSO_4$	350 °C	22.30c	24.73a	25.00a
Biochar + $FeSO_4$	550 °C	24.13ab	19.03ef	23.33bc
Biochar + Dolomite	350 °C	25.23a	25.07a	18.67f
Biochar + Dolomite	550 °C	24.63a	24.57a	19.23ef

Notes: Numbers followed by the same letter in the same row, column, and in the same week of observation show no significant difference according to the honestly significant different test (HSD) at α=5%.

RESULTS

The results showed significant differences existed between the control treatment (without biochar) and with biochar application for the number of tillers per plant from observations made at 2, 4, 6, and 8 weeks after of planting in rice (*O. sativa* L.) genotypes (Table 1). The interaction among the three factors, i.e., biochar modifications, pyrolysis temperature, and cultivars provided a significant effect on the number of tillers per plant from observations made at 2, 6, and 8 weeks after planting.

Table 2. Number of productive tillers, flowering age, and harvesting age of three rice cultivars with biochar treatments.

Biochar modifications	Pyrolysis Temperature	Cultivars		
		IF-16	Inpari-32	Ciherang
	Number of productive tillers (stems)			
Control (without biochar)		12.40cd	11.23d	7.23e
Biochar	350 °C	12.93c	13.30b	12.30c
Biochar	550 °C	14.17b	15.47b	13.23bc
Biochar + Fe_3O_4	350 °C	18.50a	18.97a	19.63a
Biochar + Fe_3O_4	550 °C	18.90a	20.20a	20.27a
Biochar + $FeSO_4$	350 °C	19.67a	18.93a	19.30a
Biochar + $FeSO_4$	550 °C	20.13a	19.83a	20.13a
Biochar + Dolomite	350 °C	18.27a	18.10a	18.30a
Biochar + Dolomite	550 °C	19.07a	19.47a	19.23a
	Flowering age (days)			
Control (without biochar)		49.70j	48.93j	49.73j
Biochar	350 °C	51.23i	51.67i	51.23i
Biochar	550 °C	54.80fg	52.13hi	53.80g
Biochar + Fe_3O_4	350 °C	57.87abc	58.57a	56.90b-e
Biochar + Fe_3O_4	550 °C	56.67cde	57.67abc	58.37a
Biochar + $FeSO_4$	350 °C	55.57ef	55.63ef	57.43abc
Biochar + $FeSO_4$	550 °C	57.83abc	55.63ef	57.33a-d
Biochar + Dolomite	350 °C	58.13ab	58.33a	57.97abc
Biochar + Dolomite	550 °C	58.60a	59.00a	55.77ef
	Harvesting age (days)			
Control (without biochar)		86.17g	87.73g	86.87g
Biochar	350 °C	90.20f	90.10f	91.50ef
Biochar	550 °C	90.50f	90.43f	91.17f
Biochar + Fe_3O_4	350 °C	91.17f	90.43f	94.87abc
Biochar + Fe_3O_4	550 °C	93.60cde	91.47ef	94.13bcd
Biochar + $FeSO_4$	350 °C	95.17abc	92.20def	91.27f
Biochar + $FeSO_4$	550 °C	95.10abc	94.80abc	95.97ab
Biochar + Dolomite	350 °C	92.13def	93.70b-e	94.60abc
Biochar + Dolomite	550 °C	93.67b-e	94.43a-d	96.50a

Notes: Numbers followed by the same letter in the same row, column, and in the same parameter show no significant difference according to the honestly significant different test (HSD) at α=5%.

The results revealed biochar application treatments manifested a considerable increase in the number of tillers compared with the control (Table 1). The decrease in growth traits of the control treatment (without biochar) was due to the disruption of physiological processes due to mercury exposure. The same pattern was also evident in the number of productive tillers (Table 2), and a substantial increase in the number of productive tillers emerged with the biochar treatment, and the same was more prevalent in the modified biochar treatment. However, overall, a nonsignificant difference occurred among the modified biochar treatments.

The mercury content analysis indicated mercury contents were above the threshold level in rice roots, stems, leaves, and grains in the treatment without biochar (Figure 2). The average mercury content in rice roots, stems, leaves, and grains in no biochar treatment were 0.08, 0.06, 0.06, and 0.05 mg kg^{-1}, respectively. Wu *et al.* (2018) also reported rice produced in Hg mining areas contained a mercury level of above 100 µg kg^{-1}. However, the biochar treatments showed a significant decrease in mercury content in rice roots, stems, leaves, and grains.

Figure 2. Mercury content in a) roots, b) stems, c) leaves, and d) rice grains.

Table 3. Number of filled grains, empty grains, and production per plot of three rice cultivars with biochar treatment.

Biochar modifications	Pyrolysis Temperature	Cultivars		
		IF-16	Inpari-32	Ciherang
	Number of filled grains (grains)			
Control (without biochar)		125.33b	60.67bc	33.67c
Biochar	350 °C	169.00a	174.00a	224.67a
Biochar	550 °C	178.33a	175.67a	209.33a
Biochar + Fe_3O_4	350 °C	160.00a	183.67a	199.33a
Biochar + Fe_3O_4	550 °C	176.00a	211.00a	210.33a
Biochar + Sulfur	350 °C	189.33a	191.67a	215.00a
Biochar + Sulfur	550 °C	208.00a	164.00a	201.67a
Biochar + Dolomite	350 °C	206.67a	239.33a	204.33a
Biochar + Dolomite	550 °C	224.33a	219.00a	225.67a
	Number of empty grains (grains)			
Control (without biochar)		91.67b	129.00c	133.00c
Biochar	350 °C	57.67a	58.67a	45.33a
Biochar	550 °C	55.67a	58.33a	53.00a
Biochar + Fe_3O_4	350 °C	33.33a	36.33a	41.33a
Biochar + Fe_3O_4	550 °C	45.33a	53.67a	56.00a
Biochar + Sulfur	350 °C	37.33a	57.67a	43.00a
Biochar + Sulfur	550 °C	42.00a	47.33a	48.33a
Biochar + Dolomite	350 °C	44.67a	46.33a	49.00a
Biochar + Dolomite	550 °C	47.33a	41.67a	43.33a
	Production per plot (gram)			
Control (without biochar)		558.00kl	432.00m	434.00m
Biochar	350 °C	618.00h-l	575.33jkl	535.33l
Biochar	550 °C	742.00b-e	638.00f-k	625.33g-l
Biochar + $Fe3O_4$	350 °C	934.00a	807.33bc	724.67c-f
Biochar + $Fe3O_4$	550 °C	956.00a	833.33b	774.00bcd
Biochar + Sulfur	350 °C	718.00c-g	619.33h-l	658.00e-j
Biochar + Sulfur	550 °C	794.67bc	588.67i-l	673.33e-i
Biochar + Dolomite	350 °C	616.67h-l	624.67g-l	597.33h-l
Biochar + Dolomite	550 °C	648.00e-k	638.00f-k	687.33d-h

Notes: Numbers followed by the same letter in the same row, column, and in the same parameter show no significant difference according to the honestly significant different test (HSD) at α=5%.

The negative effects on rice plants after exposing to mercury are visible in the increased number of empty grains and the decrease in the number of filled grains (Table 3). The highest increase in production resulted in the biochar treatment modified with Fe_3O_4. The average increase in production due to unmodified biochar treatment was 22.4% at 350 °C pyrolysis temperature and 41.6% at 550 °C temperature. Meanwhile, average increases in modified biochar treatment with Fe_3O_4 were 73.7% at 350 °C pyrolysis temperature and 80.9% at 550 °C temperature. With the modified biochar treatment with $FeSO_4$, these were 41.2% at 350 °C pyrolysis temperature and 44.6% at 550 °C temperature, while with the modified biochar treatment with dolomite was 30.9% at 350 °C pyrolysis temperature and 40.7% at 550 °C temperature. The results showed, in addition to the highest increase in production with biochar treatments modified with Fe_3O_4, it was apparent that the upsurge in rice production occurs due to a rise in pyrolysis temperature.

DISCUSSION

The decrease in plant growth traits in the treatments without biochar application occurred due to the presence of mercury, which caused disruption in nutrient absorption and photosynthesis processes. The imbalance of mercury content in photosynthetic cells caused inhibition of the photosynthetic process by disrupting the carbon reaction (Shomali *et al.,* 2024). Rising heavy metals beyond the threshold also hinders water absorption and homeostasis processes (Saini *et al.,* 2021). The heavy metals in the soil also upset the status of nutrients in the soil (Bath *et al.,* 2019), disrupting the absorption of other nutrients needed by crop plants, especially the potassium element (Asgher *et al.,* 2024).

The channels for Hg entry into plants are the same as those for K+ entry (Sestak *et al.,* 2022). Biochemically, plants have a natural defense against heavy metal stress by producing specific compounds. The compound produced by crop plants to inhibit the entry of Hg into plants is tetraethylammonium (TEA). The TEA compound will close the Hg entry channel, and at the same time, will close the entry of K+ in crop plants, which can cause K deficiency in them (Kudryashova *et al.,* 2021). The K deficiency in plants will cause disruption of anion and cation balance, and the protein synthesis and enzymes act as stress response in the formation of antioxidant ascorbic acid (De-Luca *et al.,* 2021; Johnson *et al.,* 2022).

A decrease in the flowering age and harvesting age resulted in the treatments without biochar. This also indicates the plants were under mercury stress conditions. Plants experiencing stress conditions, including mercury stress, will try to avoid pressure conditions, including shortening plant life, accelerated senescence, and leaf abscission as the plant's 'escape strategies' against stressful settings (Kooyers, 2015). Sade *et al.* (2018) reported in crop plants, the stress conditions can accelerate the senescence process. As a result of the senescence process in plants, it will have an impact on the disruption of the photosynthesis process, which has implications for reducing yield.

The modified biochar application has a considerable role in reducing the mercury content. For rice grain mercury content, all the biochar treatments have proven capable to reduce mercury content and keep below the threshold level, except the rice cultivar Ciherang. The results indicated interaction effects between the biochar modification and rice cultivars, and the cultivar Inpari-32 appeared as moderately resistant to mercury. Past findings revealed biochar application with other materials can significantly reduce the mercury content in rice grains, and the accumulation of MeHg in rice grains decreased by 50% and 70% after adding biochar – Se co-application (Wang *et al.,* 2019).

The ability of the modified biochar to reduce mercury uptake in rice plants has a link to the pore area of the biochar surface and the groups formed through the modification process. Modification with Fe_3O_4 at different temperatures causes differences in biochar pore area and volume. Pyrolysis temperature of 550 °C causes the area and pore volume of biochar to increase. The presented results were greatly analogous to the findings of Giwa *et al.* (2022), who suggested that at pyrolysis temperatures ranging from 400 °C to 900 °C, the surface area of biochar begins to increase because the micropore structure of biochar simultaneously begins to form. In contrast to the latest results, Chaijak *et al.* (2023) reported the low pyrolysis temperature at 300 °C was effective in removing Pb metal by 96.10% ± 0.30%.

Biochar modification with Fe_3O_4, $FeSO_4$, and dolomite promotes pore development and leads to the expansion of pore volume and increase in surface area. Dou and Jiang (2019) suggested the modified biochar has a higher surface area. Zhang *et al.* (2019) used the sewage sludge biochar and reported an increase in methyl mercury in soil; however, a decrease of 73% appeared in methyl mercury in rice grains. Methyl mercury is the form of mercury that is most easily translocated to plant parts.

Applying unmodified and modified biochar decreased the number of empty grains, increased the number of filled grains and grain

Table 4. Soil pH at the beginning and end of the study.

Treatments	Soil pH	
	Start of research	End of research
Control (without biochar)	5.4	5.3
Biochar (350 °C)	5.4	5.6
Biochar (550 °C)	5.4	5.8
Biochar (350 °C) + Fe_3O_4	5.4	6.4
Biochar (550 °C)+ Fe_3O_4	5.4	6.7
Biochar (350 °C) + $FeSO_4$	5.4	4.6
Biochar (550 °C) + $FeSO_4$	5.4	4.6
Biochar (350 °C)+ Dolomite	5.4	6.7
Biochar (550 °C) + Dolomite	5.4	6.8

yield per plot. The presence of mercury metal in plant roots can reduce the uptake of K metal for plants. For plants, the most common symptoms of mercury exposure are root growth inhibition (Wang *et al.,* 2013, 2018), photosynthesis inhibition and yield reduction (Murugaiyan *et al.,* 2019), disruption of water transport and uptake systems, and leaf chlorosis (Liu *et al.,* 2021), and inhibition of potassium uptake (Sestak *et al.,* 2022). The modification of biochar with Fe_3O_4 will produce magnetic biochar (Liang *et al.,* 2021). Reguyal and Samah's (2018) findings showed magnetic biochar is stable and works efficiently in the pH, ranging from 4.5-8.5. Tomczyk *et al.* (2020) reported biochar pyrolysis at 350 °C–700 °C has a higher pH than the biochar made at a lower pyrolysis temperature.

Biochar modified with $FeSO_4$ proved effective in reducing mercury content in plants; however, in production, it has no significant difference with the biochar without modification. The results showed adding $FeSO_4$ to biochar can produce sulfhydryl groups that can improve its adsorption performance. Lu *et al.* (2023) reported the maximum adsorption capacity of sulfhydryl modified biochar was about 1.6 times higher than the biochar without modification. The rapid effect of sulfhydryl modification provides additional functional groups and improves the chemical sorption and physical adsorption properties. Nordberg *et al.* (2015) suggested molecules with sulfhydryl groups are indicative as mercury. The insignificant increase in production in the biochar treatment modified with $FeSO_4$ correlated with the decrease in soil pH, which can cause a disruption of nutrient uptake. However, in other treatments, the biochar application emerged effective in increasing the soil pH (Table 4). Tasnim *et al.* (2021) also reported the use of biochar combined with vermicompost can increase the soil pH compared with the vermicompost application alone.

The mercury content decrease in biochar application referred to the biochar's ability to bind mercury and sulfhydryl groups, as well as, the magnetic biochar providing a favorable residence for soil microorganisms, enabling some to absorb toxic metals biologically, including Hg. Giovanellaa (2017) reported the *Pseudomonas sp.* isolate B50D showed the best performance in removing mercury. Cabral *et al.* (2016) also declared the ability of *Pseudomonas putida* in degrading methylmercury.

CONCLUSIONS

The results suggested the modification of biochar with Fe_3O_4, $FeSO_4$, and dolomite can reduce the rice (*O. sativa* L.) grain's mercury content below the threshold level. The interaction of rice cultivar IF-16 and biochar modification with Fe_3O_4 proved effective in reducing mercury content and increasing rice production.

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